Bacterial Volatiles Mediating Information Between Bacteria and Plants

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Abstract At present, more than 400 volatiles are known to appear in bacterial headspace samples, but more are expected as more bacteria will be investigated and several identification technologies will be applied. A comprehensive list of bacteria and their respective effects on plants were presented. The volatiles emitted from *Serratia plymuthica* HRO-C48 and *Stenotrophomonas maltophilia* R3089 retarded leaf and root development of *Arabidopsis thaliana* starting at day 2 of cocultivation, while first signs of activation of stress promoters appeared already after 18 h. Most *A. thaliana* ecotypes reacted similar to the volatiles of *S. plymuthica*, but a stronger root growth inhibition was observed for the accession C24. β-Phenyl-ethanol was identified as one compound of the *S. plymuthica* volatile mixture inhibiting the growth of *Arabidopsis thaliana*.

1 Introduction

Most of the compounds of fragrances known today originate from plants and animals. It is not commonly realized that also prokaryotes produce and emit an enormous diversity of volatiles, although the aromas of cheese and wine are well known (e.g., Urbach 1997; Schreier 1980). Furthermore, it is not very evident that the earthy smell in forests is primarily due to the emission of volatiles synthesized

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by bacteria, e.g., actinomycetes emit the typical earth odor geosmin (Gerber and Lechevalier 1965). Volatiles are chemicals with low molecular masses (<300 Da), low polarity, and high vapor pressure (0.01 kPa or higher at 20°C). Together, these features facilitate evaporation. Typical volatiles are monoterpenes, aromatic compounds, and fatty acid derivatives. They appear in the atmosphere and act over long distances. Besides aboveground volatile-based exchanges, also below-ground volatile interactions have to be considered. The biological and ecological roles of bacterial volatiles were so far underestimated, and it is a future task to unravel their action potentials. In this chapter, we focus on the interactions between bacteria and plants that are solely based on volatile compounds; bacterial interactions based on nonvolatile metabolites were not considered. The latter activate plant defense mechanisms and stimulate signal transduction pathways, such as SAR (systemic acquired resistance) or ISR (induced systemic resistance) with salicylic acid and jasmonic acid as key components. It is a goal of upcoming research to unravel whether and which responses or signaling chains are activated in plants after bacterial volatile perception. The processes of volatile perception and the conversion of information remain so far elusive.

This chapter describes first the state of the art regarding the wealth and distribution of bacterial volatiles including information about collection and detection. Thereafter, the cellular and molecular alterations in plants due to bacterial volatile administration are addressed. Finally, an ecological aspect was taken into consideration.

2 The Wealth of Bacterial Volatiles

Microorganisms, including bacteria, are everywhere on the earth, in the air, in the water, in the soil, in extreme localizations (in hot springs, in arctic regions, several 1,000 m deep in the ocean), as well as in and on organisms. They produce a large spectrum of volatiles, inorganic as well as organic compounds. Often, these volatiles contribute to the characteristic aroma of foodstuffs, such as vine and beer, cheese and other milk products, sour cabbage, or other fermented eatables. The qualitative and quantitative volatile compound compositions of aromas are primarily determined by the bacterial species and their growth conditions. The availability of substrates and the metabolic capabilities and capacities of the bacteria are decisive for product formation, including volatile emission (Stotzky and Schenck 1976; Fiddaman and Rossall 1994).

The first publication that indicated the emission of volatile fatty acids from *Dysenteria* bacteria appeared in 1921 (Zoller and Mansfield Clark 1921). Our recent literature search included 336 bacterial species that produce volatile organic compounds (VOCs). In total, ca. 770 different VOCs are released by bacteria. These compounds were grouped into ca. 50 classes, such as acids, alcohols and aldehydes (Fig. 1). The dominant compound groups were alcohols, alkenes, ketones, and terpenoids (comprising 120–190 different substances) followed by acids, benzenoids, esters, or pyrazines (comprising 60–80 different compounds),

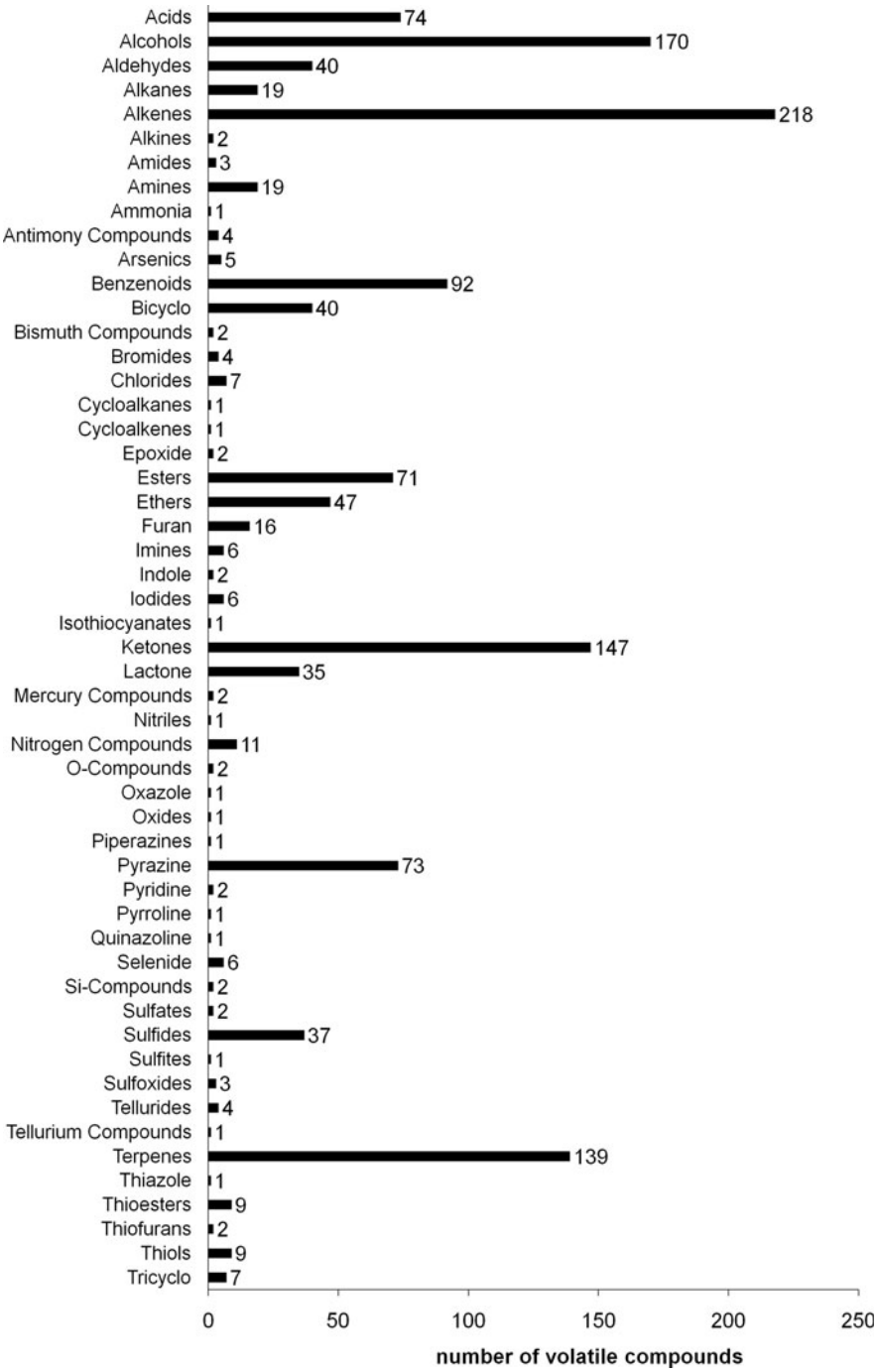

Fig. 1 Distribution of bacterial volatiles in chemical classes**.** Presently known bacterial volatiles are assigned to different chemical classes

and aldehydes, ethers, and lactones (comprising 30–40 compounds). *Chondromyces crocatus*, *Carnobacterium divergens* 9P, *Streptomyces* sp. GWS-BW-H5, and *Serratia odorifera* 4Rx13 are the bacteria with the largest VOC emission spectra, ca. 75–100 compounds were emanated by each species (Schulz et al. 2004; Ercolini et al. 2009; Dickschat et al. 2005; Kai et al. 2010). Seven hundred seventy bacterial VOCs were incorporated into the SuperScent database, which is open for public access (http://bioinf-applied.charite.de/superscent/ index.php?site = home). Besides the VOCs with identified structure, numerous bacterial volatiles and their isomers remain to be structurally elucidated. Recently, we successfully isolated and characterized a new compound from *Serratia odorifera* 4Rx13 (Kai et al. 2010). Its extraordinary chemical structure is new to science, and it was named "sodorifen" (von Reuß et al. 2010).

The VOC profiles of ca. 340 bacterial strains were analyzed so far, which represent a rather small number compared to species and isolates existing on earth. Therefore, more VOC spectra from prokaryotes need to be investigated in the future to identify and estimate the potential of these natural compounds. To define the VOC spectra of bacteria as complete as possible, several methods have to be applied.

3 Methods to Collect and Detect Volatiles

The techniques described below are suitable to collect and investigate volatiles, which are emitted into the headspace of bacterial cultures. Bacterial volatiles can be captured in open or closed airflow systems. The volatiles of this dynamic headspace are trapped on polymeric adsorption matrices (SuperQ, Tenax, Lewatit, and activated charcoal). In open volatile collection systems (Ryu et al. 2003; Kai et al. 2007; Kai et al. 2010), purified, sterile air enters the test vessel. Half of the influx air is sucked out and is delivered to an adsorption trap; consequently, a defined volume of excess air escapes. Therefore, external gaseous compounds and bacterial contaminations can be avoided. In closed systems, the total headspace air is analyzed since the airflow circulates continuously through the bacterial culture and through the trap (e.g., Dickschat et al. 2004; Schulz et al. 2004). This "closed-loop-stripping apparatus" (CLSA) was established by Boland et al. (1984). An alternative without continuous airflow is the analysis of the waste air of a bioreactor containing *Streptomyces citreus* by direct adsorption on a Lewatit-filled glass tube (Pollak et al. 1996). Compounds trapped in open or closed systems are either eluted with a solvent (methanol, dichloromethane, pentane) and analyzed using gas chromatography/mass spectrometry (GC/MS) or directly thermally desorbed.

Another possibility to extract bacterial volatiles encounters the static headspace of bacterial cultures using solid-phase microextraction (SPME). SPME was introduced in 1990 (Arthur and Pawliszyn 1990). A thin film of an extracting phase immobilized over the surface of a fused silica fiber facilitates the adsorption of compounds present in the headspace. According to the properties of expected volatiles, different coatings

are available for extraction, e.g., polydimethylsiloxan, carboxen, and divinylbenzene or combinations of these adsorbents. The SPME technique provides advantages, e.g., the method is solventless, simple in situ sampling, and a short analytical time. Till now, several bacterial headspace-SPME investigations have been performed (e.g., Vergnais et al. 1998; Kataoka et al. 2000; Chuankun et al. 2004; Schulz et al. 2004; Farag et al. 2006; Zou et al. 2007; Ercolini et al. 2009; Preti et al. 2009). Other static approaches (diffusive sampling) were established (Larsen and Frisvad 1994) using polymeric substances (Carbon black, Tenax). They were filled into stainless steel tubes and directly placed into the Petri dishes to capture volatiles from the headspace of different bacterial cultures (Schöller et al. 1997), or activated charcoal was placed in the lid of the Petri dishes (Gust et al. 2003).

All volatile collection methods mentioned above were combined with GC/MS techniques. Instead of GC/MS, the collection system can also be attached to proton transfer reaction/mass spectrometer (PTR/MS) (Mayr et al. 2003; Bunge et al. 2008; Kai et al. 2010) or selected ion flow tube/mass spectrometer (SIFT/MS) (Carrol et al. 2005; Allardyce et al. 2006; Thorn et al. 2010). While GC/MS depicts volatile profiles that are based on the analyses of defined retention times, PTR/MS and SIFT/MS allow continuous monitoring of volatile emission. Another substantial benefit of PTR/MS and SIFT/MS is that prior to analysis no preconcentration step or chromatography is needed. PTR/MS determines the *m*/*z* ratio of a molecule and no fragmentation pattern; therefore, the use of natural isotopic ratios and literature search are necessary to make an educated guess to identify the compounds. To overcome this limitation, an alternative method can be used to detect and characterize volatiles: secondary electron spray ionization/mass spectrometry (SESI/MS) (Zhu et al. 2010). It has to be realized that all specific techniques mentioned here only allows the detection and determination of a certain spectrum of volatiles emitted from the bacteria. To get a comprehensive compilation of volatiles, it is inevitable to combine the different volatile collection methods.

4 Bacterial Volatiles Mediating Interactions with *Arabidopsis thaliana*

4.1 Observations at the Level of Phenotype

In contrast to the large number of bacterial volatiles that have been described so far, not many details are known about their ecological and biological functions. This issue is difficult to approach because bacterial volatiles can act as individual compounds or in mixtures of different compositions. Another drawback is that often the complete volatile spectra of bacteria are not known, or the contributions of individual compounds in mixtures have yet not been determined. Furthermore, the biologically active compound(s) and relevant concentration(s) are not known. Dual cultures where only volatiles can act as a functional agents are simple test systems.

In one compartment of bipartite or tripartite Petri dishes, bacteria were plated, and in the other compartment(s), young plant seedlings, (*Arabidopsis thaliana* or *Physcomitrella patens*) were planted. Only volatiles can diffuse through the atmosphere from one side to the other side of the Petri dish. The growth of the plants during cocultivation was followed by photographic documentation or determination of, e.g., fresh weight, leaf length, or root length. Figure 2a summarizes the experiment performed with the volatiles of 11 bacterial strains and isolates acting on *A. thaliana* (Vespermann et al. 2007; Kai et al. 2008). While *A. thaliana* develops normally in coculture with *Bacillus subtilis*, *Burkholderia cepacia*, *Staphylococcus epidermidis*, and *Escherichia coli*, weak growth or no growth was obtained with *Pseudomonas fluorescens*, *Pseudomonas trivialis*, *Serratia odorifera*, *Serratia plymuthica*, *Stenotrophomonas maltophilia*, and *Stenotrophomonas rhizophila*. Phenotypical changes that appeared during cocultivation with S. *plymuthica* HRO-C48 and *S. maltophilia* R3089 were visible after 5 days (Fig. 2c). Dual culture assays with application of increasing cell numbers of *S. plymuthica* HRO-C48 (Fig. 2b) resulted in significant effects on green plant parts and roots. The more bacterial cells were applied at the beginning of the experiment, the more dramatic phenotypic effects were observed at *A. thaliana*. A stronger effect on the relative root growth could be observed compared to the inhibition of cotyledons. This difference between the effects on belowground and aboveground plant parts is presumable due to faster elongation growth of root cells. It also should be considered that the diffusion of volatiles is different in the agar versus in the air of the Petri dish; it is a consequence of different polarity and volatility of individual compounds. Also, the mode of perception as well as the mode of action *in planta* (direct or indirect) is until now an open question. The presented experiments, however, clearly demonstrate that the highest tested number of 10^7 CFU of *S. plymuthica* HRO-C48 caused significant retardation of root and leaf growth within 2 days of cocultivation. These cell numbers are ecologically relevant because at strawberry roots under field conditions, *S. plymuthica* HRO-C48 reached up to 10^7 CFU per g (Kurze et al. 2001), and in potato and oilseed rape rhizospheres, 10^8 CFU per g root fresh weight was determined (Berg et al. 2002). Furthermore, formation of microbial biofilms on root surfaces was also reported with locally high densities of rhizobacteria (Bloemberg et al. 2000; Bais et al. 2004; Walker et al. 2004).

4.2 Alterations at the Physiological and Molecular Level

Exposure to bacterial volatiles resulted in phenomenological alterations, which are the most likely consequences of changes at the cellular and physiological levels. Cotyledons of seedlings of *A. thaliana* were incubated with Evans blue dye, which is an indicator for cell vitality. The blue color accumulates only in dead cells without intact cellular membranes (Kim et al. 2003). Leaf growth arrested between the third and fourth day in dual culture of *S. maltophilia* R3089 and *S. plymuthica* HRO-C48 (Fig. 2b). In the same time frame, Evans blue staining leads to weak local

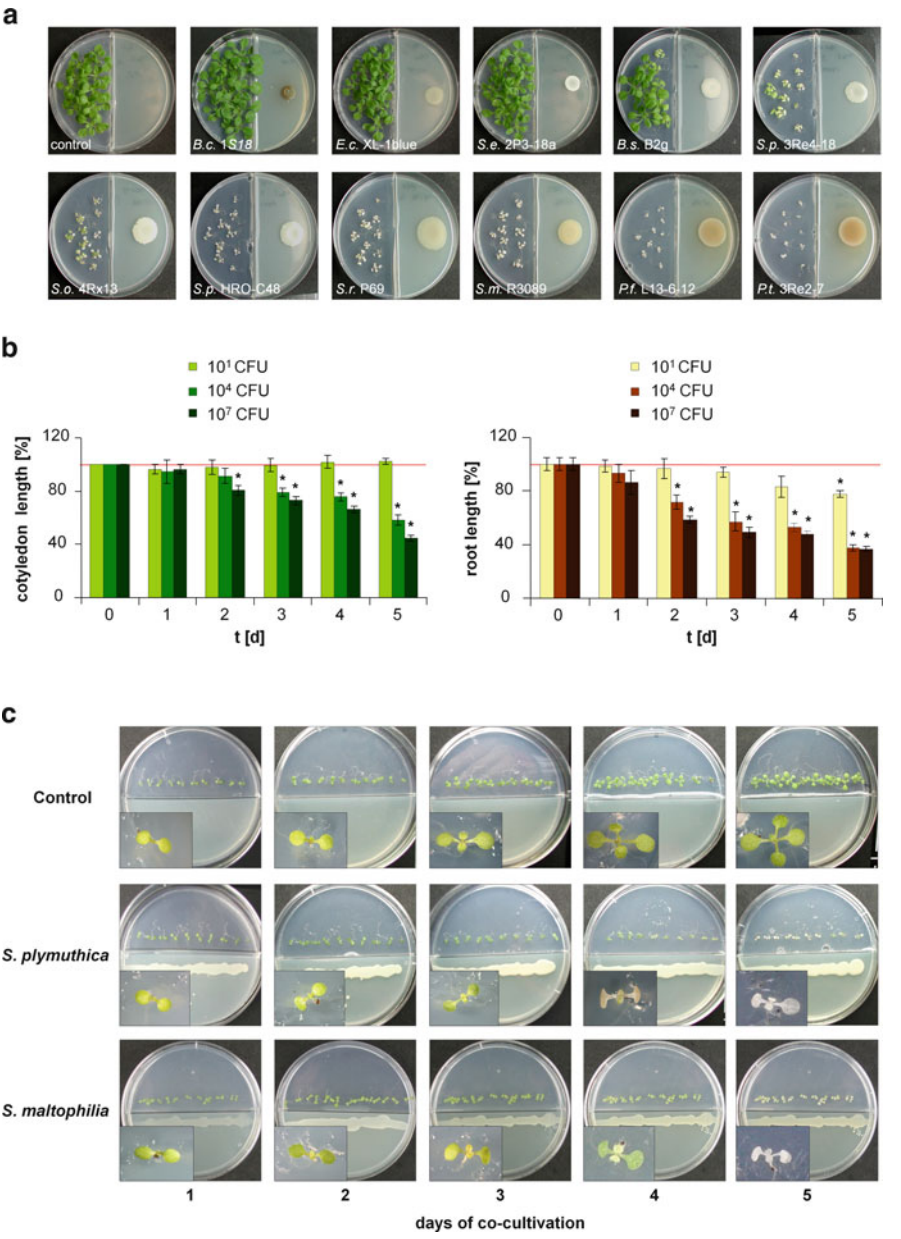

Fig. 2 Bacterial volatiles affect the growth of plants. (**a**) *Arabidopsis thaliana* in coculture with several rhizobacteria (*Bacillus subtilis* B2g; *Burkholderia cepacia* 1S18; *Escherichia coli* XL-1blue; *Pseudomonas fluorescens* L13-6-12; *P. trivialis* 3Re2-7; *Serratia odorifera* 4Rx13; *S. plymuthica* HRO-C48; *S. plymuthica* 3Re4-18; *Stenotrophomonas rhizophila* P69; *S. maltophilia* R3089; *S. epidermidis* 2P3-18a). (**b**) Bacterial cell number–dependent growth inhibitions of *Arabidopsis thaliana* cocultivated with *S. plymuthica* HRO-C48 ($n = 3$; $p \leq 0.01$), cotyledon length (*left*) and primary root length (*right*). (**c**) Photographic documentation of *A. thaliana* growth in dual cultures *S. plymuthica* HRO-C48 and *S. maltophilia* R3089 compared to control.

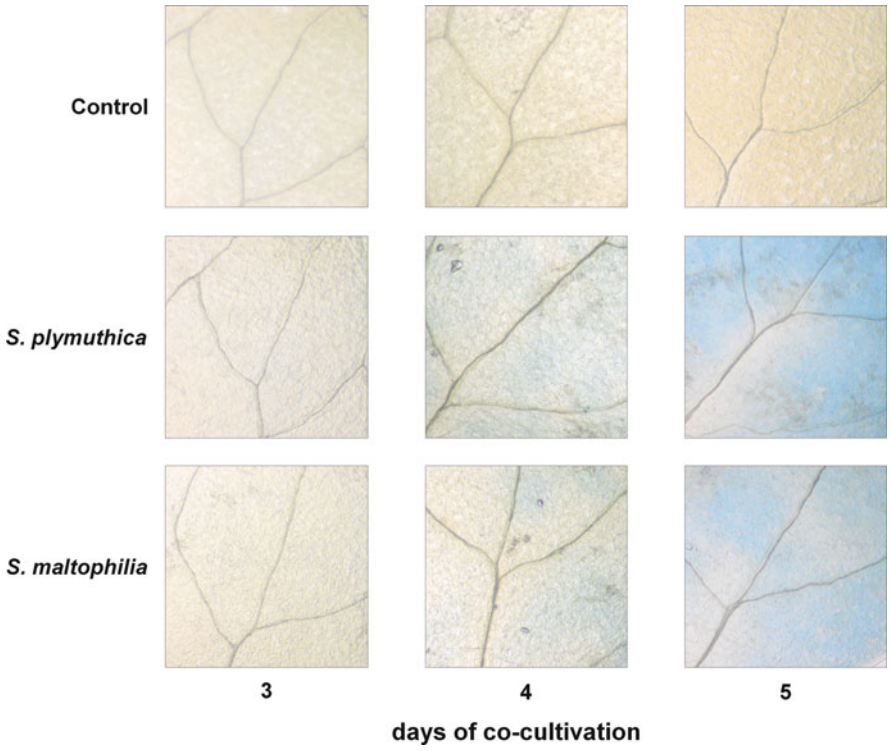

Fig. 3 Bacterial volatiles induce cell death in plants. *Serratia plymuthica* HRO-C48 and *Stenotrophomonas maltophilia* R3089 volatiles induce cell death of *Arabidopsis thaliana* cotyledons. Evans blue stains dead cells

blue signals in the cotyledons with both rhizobacteria (Fig. 3). These results show that the vitality of the leaf cells was significantly reduced after the application of volatiles of *S. maltophilia* R3089 and *S. plymuthica* HRO-C48.

These observations were further substantiated by studies at the molecular level. Synthetic plant promoter/GUS (β-glucuronidase) constructs containing defined regulatory elements (e.g., S-box, GCC-box) (Rushton et al. 2002) allow a simple and easy detection of altered gene expression due to pathogen response. The GCC-box (AGCCGCC) is often found in promoter regions of defense genes (Ohme-Takagi and Shinsi 1995), and the S-box (AGCCACC) directs gene expression upon fungal elicitor action (Kirsch et al. 2001). We used the S-box and the GCC-box promoter/GUS constructs to detect gene activation after bacterial volatile emission. Qualitative determination of the GUS activity by using 5-bromo-4-chloro-3-indolyl glucuronide (X-gluc) as substrate for the β-glucuronidase revealed an unspecific activation of the ethylene-inducible GCC-box in control experiments and *S. plymuthica* HRO-C48 cocultivated seedlings (Fig. 4a). The unregular activation/nonactivation of the GCC-box in response to the bacterial volatiles underlines the absence of ethylene in the volatile blend of *S. plymuthica* HRO-C48, which was verified by laser-based analysis

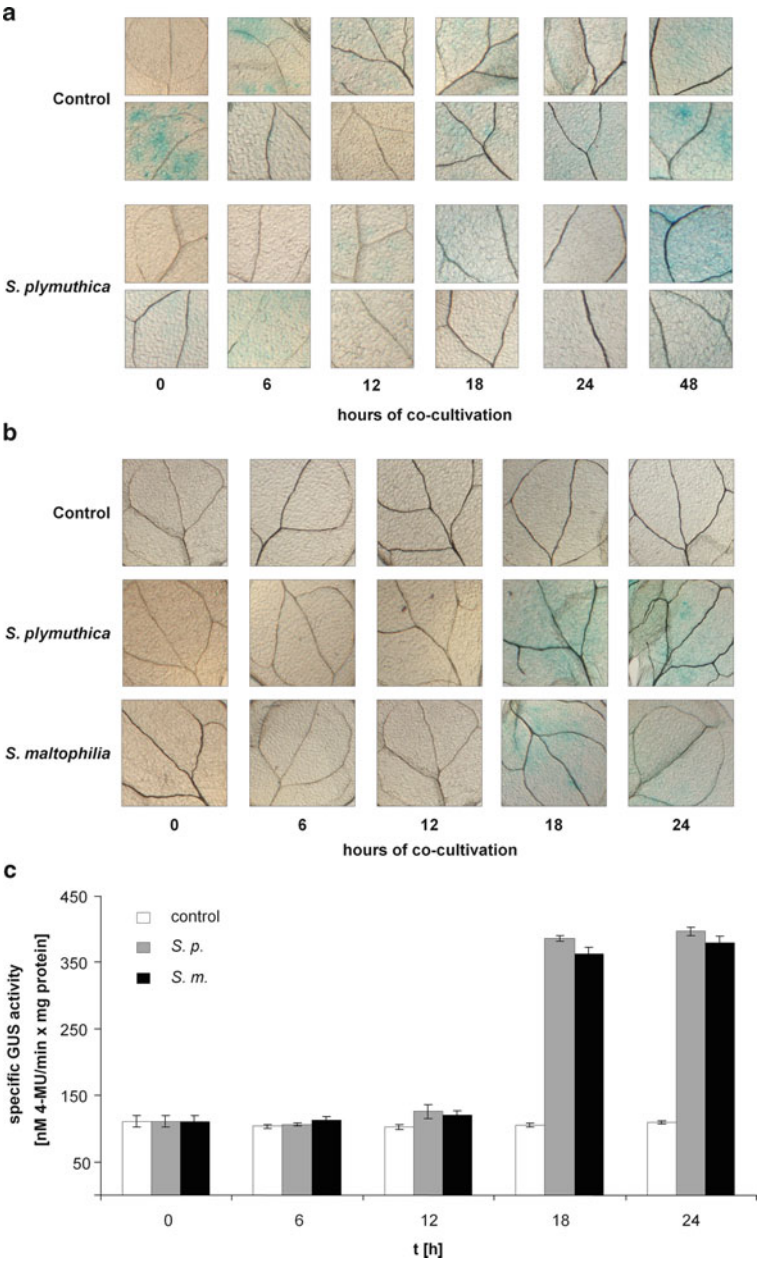

Fig. 4 Bacterial volatiles activate plant promoters. *Serratia plymuthica* HRO-C48 and *Stenotrophomonas maltophilia* R3089 activate stress-inducible promoter elements fused to the β-glucuronidase (GUS) marker gene. Induction of GUS gene expression is visualized by formation of a blue product of degraded 5-bromo-4-chloro-3-indolyl glucuronide (X-Gluc). (**a**) Activation of the GCC-box in *Arabidopsis thaliana* cocultivated with the rhizobacterial strain *S. plymuthica* HRO-C48 compared to control. (**b**) Activation of the S-box in *A. thaliana* cocultivated with the rhizobacterial strain *S. plymuthica* HRO-C48 and *S. maltophilia* R3089 compared to control. (**c**) Quantification of S-box-dependent GUS activity with 4-methylumbelliferyl-β-D-glucuronide (MUG)

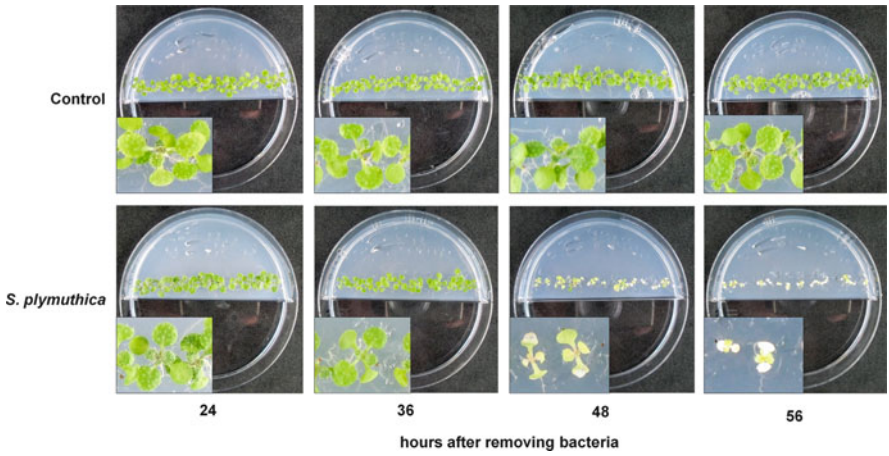

Fig. 5 Plant recovery after elimination of bacterial volatile exposure**.** Growth of *Arabidopsis thaliana* recovered after removal of *Serratia plymuthica* HRO-C48 within 36 h of cocultivation. Longer periods of cocultivations (>8 h) lead to growth inhibition and plant death. The growth of the seedlings was documented at day 6 after initiation of cocultivation

for the closely related *Serratia odorifera* 4Rx13 (Kai et al. 2010). In contrast, other rhizobacteria such as *Pseudomonas syringae* pv. *glycinea*, pv. *phaseolicola* (Weingart and Völksch 1997) are indeed able to produce ethylene. The promoter GUS assays with the S-box indicated volatile-dependent regulation of gene expression in dual culture with *S. plymuthica* HRO-C48 and *S. maltophilia* R3089 (Fig. 4b). The activity of the S-box/GUS element was quantitatively determined 6, 12, 18, and 24 h after initiating the cocultivation by application of bacteria (Fig. 4c). A twofold increase of GUS activity was detected 18 h after starting cocultivation. These data show that the volatiles of both bacteria have the capability to activate genes in plants via stress-responsive promoters, and furthermore, primary gene activations were detectable within one day after *A. thaliana* was exposed to bacterial volatiles.

To attest that bacterial volatiles are the causing agents, two different approaches were used. When the third compartment in a tripartite Petri dish was filled with charcoal, the plant growth could be restored because volatiles bind to charcoal (Vespermann et al. 2007). In another set of experiments, bacteria were removed after 1, 2, 3, and 4 days of cocultivation to allow recovery of *A. thaliana* (Fig. 5). The plants have the capacity to regrow when the bacteria are removed within 36 h of cocultivation. Longer exposures (48 and 56 h) to the bacterial blends dramatically reduced the recovery capacity; apparently, cell damage was too severe, and/or cell death processes had been initiated.

4.3 Bacterial Volatiles Cause Plant Growth Inhibitions

Volatiles emitted by bacteria are usually very complex mixtures (Kai et al. 2007). The observed growth promotions and inhibitions of *A. thaliana* in the dual culture assays

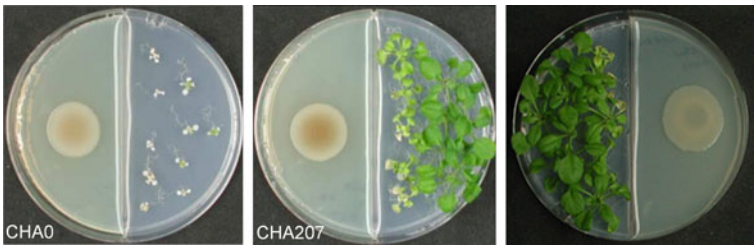

Fig. 6 Cocultivation of *A. thaliana* with *P. fluorescens* HCN-emitting CHA0 wild type (*left*) and HCN-negative CHA207 mutant (*centre*), and global regulatory CHA1144 mutant (right) strains (14 days of cocultivation)

are therefore due to the overall action of different compounds of which the causing agents and their relevant concentrations are often not known. As a first step to determine which bacterial volatiles have the potential to affect the growth, individual compounds like ammonia, HCN, and dimethyl disulfide (DMDS), were tested with *A. thaliana* (Fig. 6). Different concentrations of commercially available substances were applied on one side of the bipartite Petri dish, while *A. thaliana* was growing in the other compartment. DMDS exerts insecticidal activity via cytochrome oxidase in the mitochondrial electron transport system and potassium channel blockage (Dugravot et al. 2003; Gautier et al. 2008). The amount of DMDS with an inhibiting effect of 50% on *A. thaliana* seedlings was recently determined to be 20 µmol (Kai et al. 2010). Furthermore, Blom et al. (2011) described a negative effect of HCN on *A. thaliana* growth; 1 µmol HCN reduced plant growth ca. fourfold. Hydrogen cyanide is a volatile produced by *Pseudomonas*, *Chromobacterium*, and *Rhizobium* (Blumer and Haas 2000; Kai et al. 2010; Blom et al. 2011). The wild type of *Pseudomonas fluorescens* (CHA0) exhibited a strong volatile-dependent retardation of *A. thaliana* fresh weight, which was partially reestablished in cocultures with the HCN negative mutant *P. fluorescens* CHA207 (Fig. 6) Other volatiles than HCN also contribute to seedling growth retardation because co-cultivation with a global regulatory *P. fluorescens* mutant (CHA1144), affected in the synthesis of several secondary metabolites (Valverde and Haas 2008), fully reestablished seedling growth (Fig. 6). In addition to HCN, the CHA1144 mutant emits much less DMDS (data not shown). Additionally, reduced root length was observed in response to CHA0 and the cyanogenic *P. aeruginosa* PAO1, but no inhibition in response to respective noncyanogenic mutants (Rudrappa et al. 2008). *Serratia odorifera* 4Rx13 does not produce HCN (Kai et al. 2010), and therefore, growth inhibitions of *A. thaliana* by volatiles of *S. plymuthica* HRO-C48 also may not relate to HCN. *S. odorifera* 4Rx13, however, is able to emit ammonia at concentrations <1 µmol. At least 2.5 µmol of ammonia is necessary to inhibit plant growth in the Petri dish test system (Kai et al. 2010). A toxic effect of ammonia results in decoupling of the electron transport (Losada und Arnon 1963), which causes chlorosis and ultimately growth inhibitions (Britto und Kronzucker 2002). Ammonia and DMDS, may act additively or synergistically on plants coculturing with *S. plymuthica*. Experiments with volatile compounds applied

individually or in mixtures with different ratios need be performed in the future to understand the action potential of complex bacterial blends.

4.4 Bacterial Volatiles Cause Plant Growth Promotions

Beside bacterial volatiles exerting growth inhibitions on *A. thaliana*, also growth promotions were observed, e.g., cocultivation with *Bacillus amyloliquefaciens* IN937a and *B. subtilis* GB03 (Table 1). These bacteria are known as plant growth promoting rhizobacteria (PGPR), which support plant growth by mechanisms and agents such as (1) synthesis and release of plant hormones by bacteria (e.g., indole-3-acetic acid, cytokinin, gibberellin), (2) increasing the availability of soil minerals (e.g., Fe), (3) fixation of airborne nitrogen (N_2), and (4) release of antibiotics (e.g., antifungal metabolites AFMs), toxins, or biosurfactants (Raaijmakers et al. 2002). Bacterial volatiles apparently add another facet to the multitude of plant growth promoting mechanisms. Several publications summarized in Table 1 appeared that describe the positive growth effects in *A. thaliana* due to bacterial volatile emissions.

Bacillus subtilis GB03 is the prominent bacterium which was often used for plant growth promoting experiments. In dual culture systems, the volatile mixture of *B. subtilis* effected the auxin homeostasis; augmented photosynthetic capacity, chloroplast number, chlorophyll content, starch accumulation, and iron uptake; increased tolerance to osmotic, salt, and drought stress; reduced severity of disease symptoms; and increased resistance against pathogens of the model plant *A. thaliana* (Table 1). These induced alterations improved and stimulated the plant growth and established an additional function for volatiles as signaling molecules mediating plant-microbe interactions. The volatiles emitted by *B. subtilis* GB03 seem to influence numerous and various physiological processes. It has to be considered that GB03 emits 38 different VOCs (Farag et al. 2006). Each compound could have the potential to influence cellular or molecular processes individually. So far, only the two characteristic volatiles of bacilli, 2,3-butanediol or acetoin or the racemic mixture of 2,3-butanediol were applied individually. In these test systems, 2,3-butanediol could verify some results obtained with the bacterial volatile mixtures (leaf growth stimulation and decrease of disease symptoms); however, 2,3-butanediol was excluded to improve photosynthetic efficiency. Therefore, other compounds of the volatile blend of *B. subtilis* may be the causing agents for the latter (Farag et al. 2006). Besides the organic volatile compounds, also CO_2 emission due to metabolic reactions (e.g., tricarbonic acid cycle) has to be considered. In sealed Petri dishes, the CO_2 concentrations reached levels that were eightfold compared to ambient concentrations (3,000 ppm) (Kai and Piechulla 2009) and therefore may very well play a role in plant growth stimulations under respective test conditions. Surprisingly, out of 15 publications regarding plant growth promotions due to bacterial volatile fumigation, only one, Ezquer et al. 2010, discussed the possibility that CO_2 may affect the plants positively in the used experimental setup. Ezquer et al. (2010), however, theoretically excluded that the increased starch accumulation might be a consequence of bacterial

Table 1 Bacterial volatiles mediating plant growth promotions

Bacterial volatiles	Plants	Test system	Effects	References
B.s. GB03, *B.a.* IN937a, *B.p.* T4, *B.p.* C9, *P.f.* 89B/61, *S.m.* 90–166, *E.c.* DH5α	*A. thaliana* Col-0, C24, WS, Ler	TSA, bipartite Petri dishes sealed with parafilm	Leaf area, promotion: *B.s.*, GB03 *B.a.* IN937a	Ryu et al. (2003)
2,3-Butanediol	*A. thaliana* Col-0	TSA, bipartite Petri dish, sealed	Leaf area, promotion	Ryu et al. (2003)
B.s. GB03, *B.a.* IN937a, *B.p.* T4, *B.p.* C9, *P.f.* 89B/61, *S.m.* 90–166, *E.c.* DH5α, *E.c.* JM22, *B.p.* SE34, *B.s.* 168, *B.s.* BSIP1171	*A. thaliana* Col-0	TSA, bipartite Petri dish, sealed	*E.c.* leaf symptoms decreased, disease resistance increased: *B.s.* GB03, *B.a.*, IN937a *B.p.* T4 + C9, *P.f.*89B61	Ryu et al. (2004)
2,3-Butanediol	*A. thaliana* Col-0	TSA, bipartite Petri dish, sealed	Leaf symptoms decrease	Ryu et al. (2004)
B.s. GB03, *B.a.* IN937a, *P.f.*89B61 *E. c.* DH5α		TSA, *S. t.* (potato) slices, sealed	Volatile profiles with SPME	Faraq et al. (2006)
B.s. GB03	*A. thaliana* Col-0	TSA, bipartite Petri dish, o/c? RNA extraction 48 and 72 hours for microarray analysis	Around 350 upregulated genes, auxin synthesis upregulated, auxin accumulation down in leaves and up in roots, cell wall–loosening enzymes upregulated correlates with cell expansion	Zhang et al. (2007)
B.s. GB03	*A. thaliana* Col-0	TSA, bipartite Petri dish, o/c?	More chloroplasts, photosynthetic efficiency increased, sugar accumulation elevated, sugar sensing repressed, inhibition of hypocotyl elongation and seed germination, ABA down regulation	Zhang et al. (2008a)
2,3-Butanediol	*A. thaliana* Col-0		No effect on photosynthetic efficiency	Zhang et al. (2008a)
	A. thaliana Col-0	TSA, bipartite Petri dish, o/c? Leaf area and root mass	Salt tolerance, high-affinity K^+ transporter, HKT expression high in shoots and low in roots, lower Na^+ accumulation in whole plant	Zhang et al. (2008b)

(continued)

Table 1 (continued)

Bacterial volatiles	Plants	Test system	Effects	References
2R,3R-butanediol *P.c.* O6 and gacS mutant	*A. thaliana* Col-0, Ler, root colonization	King's medium, sealed	Induced drought resistance, decrease of stomatal apertures	Cho et al. (2008)
S.o. 4Rx13	*A. thaliana* Col-0	NB, bipartite Petri dish, sealed	Fresh weight increases due to CO_2 accumulation	Kai and Piechulla (2009)
B.s. GB03	*A. thaliana* Col-0	TSA, o/c? Magenta boxes	Growth promotion, increased inflorescences and silique number, chlorophyll, quantum yield increased, iron uptake and iron transporter upregulated	Xie et al. (2009)
B.s. GB03	*A. thaliana* Col-0	TSA, bipartite Petri dish, o/c?	Increase of iron accumulation, upregulation of Fe-deficient-induced transcription factor, root ferric reductase activity, increased acidification of rhizosphere by proton release, increased photosynthetic capacity	Zhang et al. (2009)
B.s. GB03	Ocimum basilicum	TSA, bipartite Petri dish, and Magenta boxes, o/c?	α-Terpineol and eugenol emission and essential oil increased, root and shoot biomass increase	Banchio et al. (2009)
B.s. FB17, acetoin	*A. thaliana* Col-0	LB, Magenta boxes, root inoculation, o/c?	Reduced disease severity against *P.s.* systemic resistance, ethylene-responsive gene expression increased	Rudrappa et al. (2010)
B.s. GB03	*A. thaliana* Col-0	TSA, bipartite Petri dish, o/c?	Choline and betaine synthesis enhanced, increased tolerance to osmotic stress, improved	Zhang et al. (2010)

			drought tolerance in soil-grown plants	
B.s. GB03	*A. thaliana* Col-0	TSA, bipartite Petri dish, o/c?	Proteome analysis, upregulation of ROS scavenging, ethylene biosynthesis, TCA cycle, gluconeogenesis enzymes	Kwon et al. (2010)
B.s. 168, *E. c.* BW25113, *P.s.* several isolates, *P. c.*, *P. a.*, *S. e.* LT2, *A. t.* EHA105 + GV2260	*A. thaliana* Col-0,	M9 minimal medium + 50 mM glucose, Petri dish in plastic box, o/c?	Starch accumulation on M9 medium but not on LB, consider ammonia and CO_2!	Ezquer et al. (2010)

A. thaliana Arabidopsis thaliana, *A.t.* EHA105 *Agrobacterium tumefaciens* EHA105, *A.t.* GV2260 *Agrobacterium tumefaciens* GV2260, *B.a.* IN937a *Bacillus amyloliquefaciens* IN937a, *B.p.* C9 *Bacillus pasteurii* C9, *B.p.* T4 *Bacillus pumilus* T4, *B.p.* SE168 *Bacillus pumilus* SE168, *B.s.* GB03 *Bacillus subtilis* GB03, *B.s.* 168 *Bacillus subtilis* 168, *B.s.* BSIP1171 *Bacillus subtilis* BSIP1171, *B.s.* FB17 *Bacillus subtilis* FB17, *E.c. Erwinia carotovora*, *E.c.* DH5α *Escherichia coli* DH5α, *E.c.* BW25113 *Escherichia coli* BW25113, *E.c.* JM22 *Enterobacter cloacae* JM22, *P.a. Pseudomonas aurantiogriseum*, *P.c. Pseudomonas charlesii*, *P.c.* O6 *Pseudomonas chlororaphis* O6, *P.f.*89B61 *Pseudomonas fluorescens* 89B61, *P.s. Pseudomonas syringae*, *S.e.* LT2 *Salmonella enterica* LT2, *S.m.* 90–116 *Serratia marcescens* 90–116, *S.o.* 4R×13 *Serratia odorifera* 4R×13, *S.t. Solanum tuberosum*

ROS reactive oxygen species, LB Luria-Bertani broth, TSA tryptic soy agar, ABA absisic acid, SPME solid phase micro extraction

o/c? not indicated whether open or closed (=sealed) test system was used to perform dual culture assays

CO_2 production; however, our experimental experiences with sealed plastic containers (Kai and Piechulla 2009) would argue against their theoretical considerations. Only experimental proofs could eliminate doubts.

5 Plant Volatiles Affecting *Arabidopsis thaliana* Ecotypes

Naturally occurring plant variations result from genetic diversity and epigenetic processes and occur even within one species. Besides genetic, also phenotype association studies are important to understand underlying ecological and evolutionary forces. *Arabidopsis thaliana* is an ideal candidate because of the known whole genome sequence and the availability of up to 750 accessions. It can be envisioned that *A. thaliana* had to cope with different bacterial volatile exposures under certain natural circumstances and that after long times of adaptations, different ecotypes evolved. Here we used 21 accessions of *A. thaliana* and performed cocultivations with *S. plymuthica* HRO-C48. The effects of bacterial volatiles were registered by fresh weight and root length determinations (Fig. 7a, b and c, d, respectively). The effects of *S. plymuthica* HRO-C48 volatiles on the accessions C24, Col-0, and Ler were exemplified in Fig. 7a and c. The results of all 21 accessions were summarized in Fig. 7b, d. No significant variations of fresh weight reductions (90%) were measured after exposure of the different *A. thaliana* accessions to *S. plymuthica* HRO-C48 (Fig. 7a, b). The inhibition of root growth of most accessions varied between 50% and 60%, except accession C24 (inhibition of 82%) and Ler (inhibition of 42%) (Fig. 7c, d). These results verify the higher sensitivity of primary root growth compared to the growth of green plant parts already described in Fig. 2. The hints for accession-dependent variation of root growth inhibitions correlate with experiments made by Walch-Liu et al. (2006). Concentrations of 50 μM L-glutamate lead to a similar range of inhibitions of primary roots (ca. 80% and 40% of C24 and Ler, respectively), and alterations of root branching. The latter effect was not observed in our experiments, indicating that the mode of action of L-glutamate is different to the effect of the volatiles of *S. plymuthica* HRO-C48. *A. thaliana* C24 presumably developed under laboratory conditions and Ler were isolated from the natural habitat in Landsberg (Germany). Apparently, Ler and also many other ecotypes adapted to growth inhibitions in their original locations, including to volatiles emitted by rhizobacteria, while C24 obviously did not experience such inhibitory pressures in the laboratory and therefore expresses higher sensitivity to volatiles of *S. plymuthica*.

6 Outlook

Volatile emissions of bacteria are more widespread and complex than previously thought. Comprehensive emission patterns of bacteria can only be determined when several different methods are applied and bacteria are tested under different growth

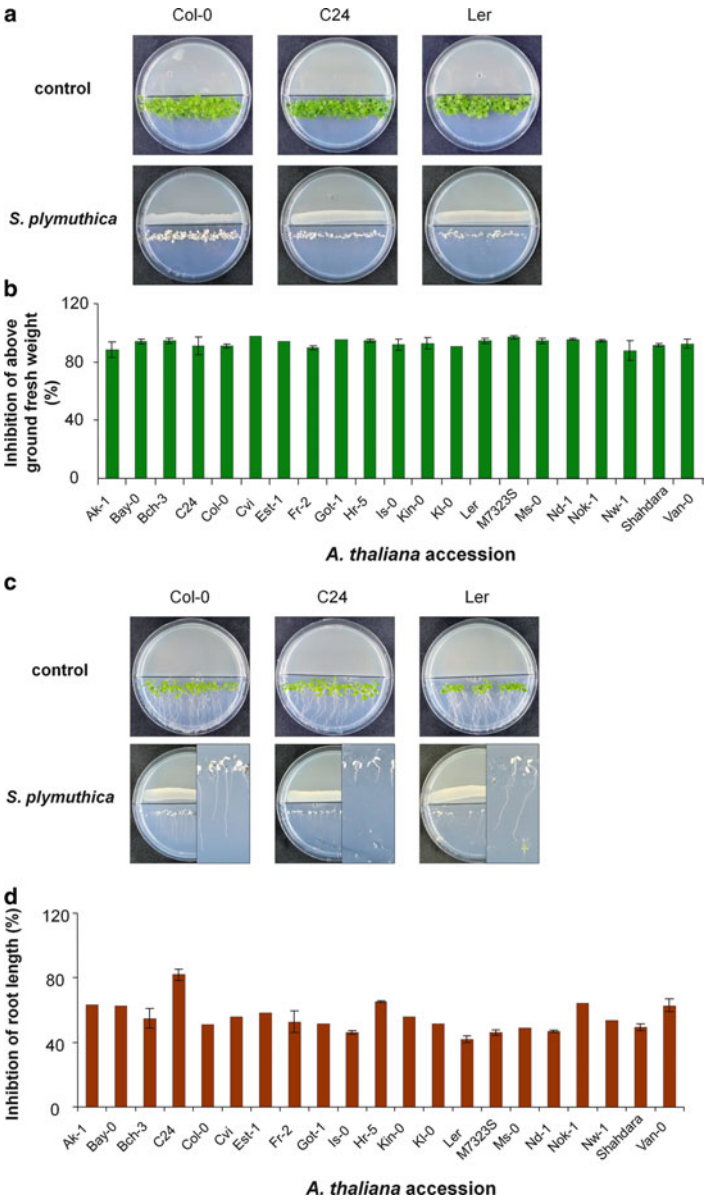

Fig. 7 Bacterial volatiles affect growth of *Arabidopsis thaliana* accessions**.** *Serratia plymuthica* HRO-C48 was cocultivated with various *A. thaliana* ecotypes. Fresh weight of aboveground plant parts (**a**, **b**) and roots (**c**, **d**) were determined after 10 days of cocultivation. Achkarren/DE Ak-1; Bayreuth/DE Bay-0; Buchen/DE Bch-3; unknown location C24; Columbia/MO Col-0; Cape Verde Islands Cvi; Estonia/EE Est-1; Frankfurt/DE Fr-2; Goettingen/DE Got-1; Isenburg/DE Is-0; United Kingdom/location unknown Hr-5; Kendallville/MI Kin-0; Kaiserslautern/DE Kl-0; Landsberg/DE Ler; unknown location M7323S; Moscow/RU Ms-0; Niederlenz/DE Nd-1; Noordwijk/NL Nok-1; Neuweilnau/DE Nw-1; Pamiro-Alay/TJ Shahdara; Vancouver/BC Van-0

conditions. It is a future research task to unravel biological and ecological effects of individual compounds as well as volatile mixtures at their relevant concentrations to elucidate the communication highway between bacteria and plants. Furthermore, it will be important to investigate the biosynthetic pathways and regulations of volatile syntheses in bacteria (emitter) and the perceptions and signal transductions in plants (receiver).

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